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Biotechnology Congress 2015 : Recombinant sFRP1 in cancer theranostics - Archita Ghoshal - Indian Institute of Technology Guwahati

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Wnt pathway is a critical aspect in the complex network of signaling molecules implicated in cancer. In non-malignant cells, Wnt signal cascade is blocked by the Secreted Frizzled-related proteins (sFRPs), a family of glycoproteins. However, they are epigenetically silenced in cancer cells. In this study, we attempted to administer recombinant human sFRP1 purified from bacterial culture on to cervical cancer cells (HeLa) to determine its anti-cancer activity. Human sFRP1 gene was cloned into bacterial expression vector pGEX-4T2. Recombinant protein was over-expressed as inclusion bodies in E.coli BL21 (DE3). After optimization of the process of solubilization of protein using detergents, GST-sFRP1 was purified to homogeneity by glutathione agarose affinity chromatography. MALDI analysis verified peptide signature particular to GST-sFRP1 and structural integrity was characterized by circular dichroism spectra. Purified recombinant protein was found to exert significant anti-proliferative effect on HeLa cells at nanomolar concentrations. Western blotting with antibodies against beta-catenin and phosphorylated beta-catenin confirmed the implication of canonical Wnt pathway. Combination therapy exhibited that recombinant sFRP1 substantially chemo sensitized HeLa cells towards conventional chemotherapeutic drug cisplatin. Coating of the therapeutic protein over gold nano-cluster embedded novel composite nanoparticles facilitated sustained release of the protein and enhanced its functional activity. The remarkable fluorescence property of gold nano-clusters was exploited for tracking and imaging purposes. Overall, therapeutic sFRP1 based nano-system may prove to be revolutionary in the field of cancer theranostics.Emitted frizzled-related protein 1 (SFRP1) is a characteristic blocker of the Wnt flagging pathway in ordinary grown-up cells however is epigenetically hushed in malignant growth cells prompting deviant expansion. In this examination, we have detailed novel composite nanoparticles manufactured with gold nanocluster inserted chitosan and alginate, bound to bacterially communicated human recombinant sFRP1. The Wnt pathway, which is upregulated in disease, has been explicitly focused with the nanoparticles to accomplish an antiproliferative impact on malignant growth cells, as clear from diminished degrees of downstream particles, to be specific, β-catenin, cyclin D1, and survivin. The nanoparticles empowered supported arrival of sFRP1 outside the cells, where it is practical. Also, amazing iridescence properties of gold nanoclusters were abused for authoritative, imaging, and following investigations. Co-treatment of sFRP1-stacked nanoparticles with the medication cisplatin focused on two free pathways to incite apoptosis, as archived by stream cytometry based examines. Generally speaking, this nanosystem is promising for following, imaging, and focusing on malignant growth motioning with restorative protein.Secreted frizzled-related protein 1 (SFRP1) is a characteristic blocker of the Wnt

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flagg sorting pathway in ordinary grown-up cells yet is epigenetically hushed in disease cells prompting abnormal multiplication. In this examination, we have announced novel composite nanoparticles created with gold nanocluster installed chitosan and alginate, bound to bacterially communicated human recombinant sFRP1. The Wnt pathway, which is upregulated in malignancy, has been explicitly focused with the nanoparticles to accomplish an antiproliferative impact on disease cells, as apparent from diminished degrees of downstream particles, in particular, β-catenin, cyclin D1, and survivin. The nanoparticles empowered continued arrival of sFRP1 outside the cells, where it is practical. In addition, surprising glow properties of gold nanoclusters were abused for authoritative, imaging, and following investigations. Co-treatment of sFRP1-stacked nanoparticles with the medication cisplatin focused on two free pathways to actuate apoptosis, as reported by stream cytometry based examines. Generally speaking, this nanosystem is promising for following, imaging, and focusing on malignant growth motioning with helpful protein.The Wnt flagging pathway assumes a dominating job in deviant multiplication in bunch of diseases. In non-carcinogenic cells, Wnts are obstructed by the emitted frizzled-related proteins (sFRPs) that are by and large downregulated in disease cells. We have refined and portrayed bacterially communicated glutathione S-transferase-labeled SFRP4 from a novel clone produced from human cell root. Cervical malignancy (HeLa) and lung disease (A549) cells, in which Wnt and related qualities were seen as communicated, were treated with the sanitized recombinant sFRP4, which uncovered a critical portion subordinate cell development hindrance up to 40 %. The present examination on usefulness of this bacterially created recombinant sFRP4 in capturing disease cell multiplication is the first of its , where G2/M stage capture and early apoptosis were clear. Increment in phosphorylated β-catenin in sFRP4 treatment showed restraint of Wnt pathway, which was additionally affirmed by downregulation of genius proliferative qualities, to be specific cyclin D1, c-myc, and survivin. Practical action of recombinant sFRP4 was additionally abused in co-treatment module with chemotherapeutic medications to disentangle atomic occasions. All in all, our investigation on cleansed recombinant sFRP4 from bacterial host holds incredible guarantee in focusing on Wnt motioning for investigating new procedures to battle malignant growth.As of late, we broke down the 8p11-12 genomic area for duplicate number and quality articulation changes in a board of human bosom disease cell lines and essential examples. We found that SFRP1 (Secreted frizzled related protein 1) is every now and again under communicated even in bosom tumors with duplicate number increments in this genomic locale. SFRP 1 encodes a WNT flagging enemy, and assumes a job in the advancement of various strong tumor types. In this examination, we broke down methylation-associated quieting of the SFRP1 quality in bosom malignant growth cells with the 8p11-12 amplicon, and explored the tumor silencer properties of SFRP1 in bosom disease cells. SFRP1 articulation was particularly decreased in both the bosom malignant growth cell lines and essential tumor examples comparative with typical essential human mammary epithelial cells in any event, when SFRP1 is enhanced. Concealment of SFRP1 articulation in bosom malignant growth cells with a SFRP1 quality enhancement is related with SFRP1 advertiser methylation. Besides, rebuilding of SFRP1 articulation smothered the development of bosom malignant growth cells in monolayer, and hindered mooring autonomous development. We likewise inspected the connection between the hushing

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of SFRP1 quality and WNT motioning in bosom malignant growth. Ectopic SFRP1 articulation in bosom malignant growth cells smothered both authoritative and non-canonical WNT flagging pathways, and SFRP1 articulation was adversely connected with the outflow of a subset of WNT responsive qualities including RET and MSX2 . Along these lines, down-regulation of SFRP1 can be activated by epigenetic or potentially hereditary occasions and may add to the tumourigenesis of human bosom malignant growth through both standard and non-canonical WNT flagging pathways. © 2009 UICC

Biography

Archita Ghoshal is currently pursuing her PhD at the Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, India. She has published two research papers and two more are in the process of being published. Her area of research encompasses molecular biology as well as nanobiotechnology. Previously, she had obtained her Bachelor's degree in Biotechnology from a reputed university in Kolkata, India. She had also participated in a Summer Project Program at the New Jersey Institute of Technology, USA, as part of a student exchange program.

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